

## Effects on the Immune System Associated with Living Near a Pesticide Dump Site

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In this paper, we report results of the second phase of a larger study designed to evaluate the effects on the immune system of living near a Superfund site containing organochlorine pesticides, volatile organic compounds, and metals. Phase II was conducted to determine whether living near the site, consisting of six locations in Aberdeen, North Carolina, is associated with higher plasma organochlorine levels, immune suppression, or DNA damage. Each of 302 residents of Aberdeen and neighboring communities provided a blood specimen, underwent a skin test, and answered a questionnaire. Blood specimens were analyzed for organochlorine pesticides, immune markers, and micronuclei. Of 20 organochlorines tested, only DDE was detected in the blood of participants (except for one individual). Age-adjusted mean plasma DDE levels were 4.05 ppb for Aberdeen residents and 2.95 ppb ( $p = 0.01$ ) for residents of neighboring communities. Residents of 40–59 years of age who lived within a mile of any site, but particularly the Farm Chemicals site, had higher plasma DDE levels than residents who lived farther away. Residents who lived near the Farm Chemicals site before versus after 1985 also had higher plasma DDE levels. Overall, there were few differences in immune markers between residents of Aberdeen and the neighboring communities. However, residents who lived closer to the dump sites had statistically significantly lower mitogen-induced lymphoproliferative activity than residents who lived farther away ( $p < 0.05$ ). Residential location was not consistently associated with frequency of micronuclei or skin test responses. Although some statistically significant differences in immune markers were noted in association with residential location, the magnitude of effects are of uncertain clinical importance. **Key words:** hazardous waste, immune system, micronuclei, organochlorines, pesticides. *Environ Health Perspect* 108:1113–1124 (2000). [Online 1 November 2000] <http://ehpnet1.niehs.nih.gov/docs/2000/108p1113-1124vine/abstract.html>

The study reported in this paper is part of a larger research study designed to evaluate the effects on the immune system of living near a National Priority List Superfund Site (the Aberdeen pesticides dumps site) located in and around Aberdeen, North Carolina, which contained organochlorine pesticides, volatile organic compounds, and metals (1). Evidence from both human and animal studies suggests that pesticides (2–4), volatile organic compounds, (5,6) and metals (7–10) are capable of adversely affecting the immune system.

The overall research study was conducted in two phases. Phase I, a telephone survey of approximately 1,600 adults, 18–64 years of age, who lived in Aberdeen and several nearby communities in southern Moore County, took place during the spring and summer of 1994. The purpose of phase I was to determine whether or not living in Aberdeen was associated with adverse effects on the immune system as indicated by the occurrence of self-reported herpes zoster and other more common infectious diseases. Results of phase I, which have been reported elsewhere (11), showed that younger residents (18–40 years of age) and residents who lived in Aberdeen before 1985 (when pesticide manufacturing plants were in operation

and before any remediation efforts took place) had a 2- to 3-fold higher risk of herpes zoster than residents of the comparison communities.

Phase II was undertaken to determine *a*) whether there was any evidence of human exposure to pesticides as the result of living near the dump site as indicated by higher levels of organochlorine pesticides in blood and *b*) whether living near the site was associated with immune suppression (as indicated by alterations in various markers of immune competence) and DNA damage (as indicated by the presence of lymphocyte micronuclei). Associations between plasma organochlorine levels and immune markers are reported in another paper (12).

The Aberdeen pesticides dumps site is officially composed of five sites located in and around the town of Aberdeen in Moore County, North Carolina. The five sites (Figure 1) include *a*) Farm Chemicals site, an abandoned pesticide manufacturing/formulating facility that operated from the mid-1930s to 1987, and the most contaminated site (with 959,500 µg/kg DDT detected in surficial soil—three times the amount at any other site; Table 1) (13); *b*) Twin site—a pesticide disposal site across the street from the

Farm Chemicals site; *c*) Fairway six, a pesticide disposal area; *d*) McIver site, a local landfill into which pesticides and drums were dumped; and *e*) Route 211 site, an old sand-mining pit into which pesticides were dumped. For purposes of this study, a sixth site, the Geigy Chemical Corporation site (also located in Aberdeen) was included. It had been the site of chemical companies and pesticide formulating companies that made DDT and other chlorinated pesticides from 1947 to 1967. Between 1967 and 1989 (when the property was abandoned), it served as a distribution site for the rebagging of prepackaged and bulk chemicals (14). Each site is approximately 1 acre in area, and all are within 2 miles of each other. Most of the sites were discovered in 1984 or 1985, when modest remediation efforts were begun (15). Extensive soil and groundwater remediation took place during the late 1990s after this study was conducted. At least 1,000 people live within a 1-mile radius of the sites (15).

In 1990, the U.S. Environmental Protection Agency (13) conducted studies showing that soil and groundwater at the Aberdeen pesticides dumps site had been contaminated with a variety of organochlorine pesticides, volatile organic compounds, and metals. About the same time, three Aberdeen municipal wells were shut down due to contamination with the organochlorine pesticide lindane. Significant airborne exposure to pesticides from the Farm Chemicals site was suspected by Robert Mobbs, a local physician, as far back as

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1948. At that time, Mobbs reported in a letter to the *Journal of the American Medical Association* (16) that a child who lived 100 yards away from the plant had died with convulsions.

In a pilot cross-sectional study conducted in the early 1990s, Backer (17) assessed effects on the immune system of pet dogs living in Aberdeen compared to those living in a control community (Pinehurst/Southern Pines, North Carolina) with no known pesticide dump sites. The 20 pet dogs that lived in Aberdeen exhibited nonstatistically significant lower CD4:CD8 ratios (an indicator of immune suppression) than the 21 control dogs ( $1.54 \pm 1.14$  vs.  $2.05 \pm 0.87$ , mean  $\pm$  SD), and statistically significant higher frequencies of micronuclei as detected in lymphocytes (number of micronuclei per 1000 binucleate cells: 24.2 vs. 11.0,  $p < 0.0001$ ).

There are many potential markers of immune suppression. In this study, we selected four major classes of markers as a screen: *a*) the absolute number and percentages of white blood cells, lymphocytes, and their subsets, *b*) the levels of different classes of immunoglobulins, *c*) the delayed-type hypersensitivity reaction to six antigens and a control as determined using the Multitest CMI skin test, and *d*) mitogen stimulation assays. These markers are broad indicators of both cell-mediated and humoral immune function, which are important in the protection from and reaction to infection as well as nonspecific resistance to infection. Classes *a-c* above include immune markers that are recommended by the National Research Council (7) as first-level tests for individuals suspected of immune deficiency. The Multitest skin test has been advocated as an alternative to intradermal skin testing because one can simultaneously apply several antigens in a standardized manner. Delayed-type hypersensitivity skin testing is an essential component of the evaluation of immune function reflecting cell-mediated immunity. Because it involves multiple steps including antigen recognition and processing, T-lymphocyte activation and response, cytokine production, and cell migration, it is thought to best represent an individual's response to exogenous antigens. The mitogen stimulation assay is an alternative to delayed-type hypersensitivity skin testing for evaluating lymphocyte function. This *in vitro* assay compares the ability of a person's lymphocytes to undergo blastogenesis in response to chemical stimulants (mitogens). The response is compared to a person's own unstimulated response and to that of known controls. Mitogens used in this study include phytohemagglutinin (PHA), concanavalin A (CON-A), and pokeweed mitogen (PWM). These mitogens represent two T-lymphocyte

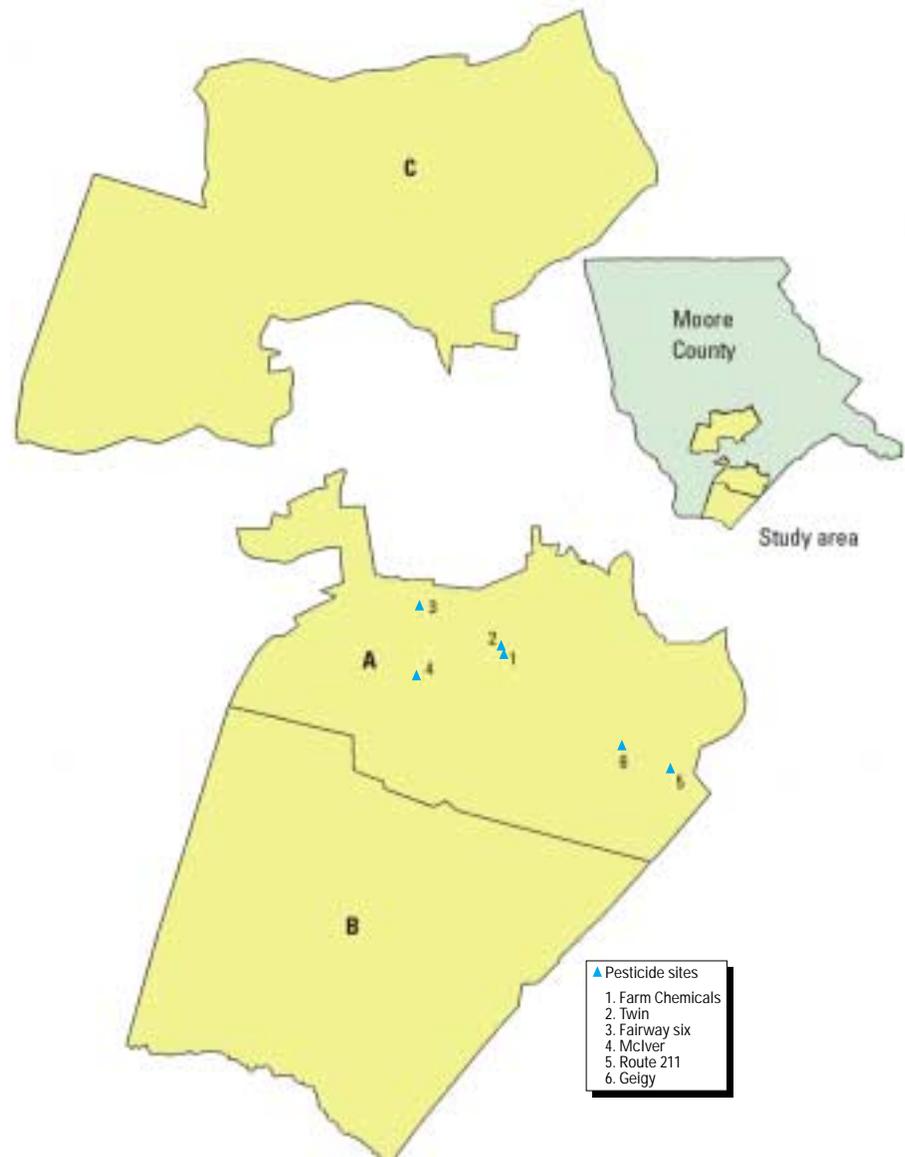
mitogens and a T-cell-dependent B cell mitogen. A positive mitogen test panel requires the functioning of both T and B cells as well as macrophages. Suppression of lymphocyte function may result in increased susceptibility to both infection and cancer. Other more specific immune assays were considered for inclusion in the study (e.g., assays for cytokines) but were not performed because of cost considerations.

## Methods

### Study Participants

Study participants included 302 adults, 18–66 years of age, who were residents of Aberdeen, North Carolina, and several neighboring communities (comparison areas). They were randomly selected (one per household) from among the 1,600 eligible

residents who participated in the phase I telephone survey study. Eligible residents included 878 residents of Aberdeen (from 614 households) and 722 residents of the comparison areas (from 524 households) (11). To be eligible for phase I, residents had to have lived for at least 1 year in Aberdeen, Pinebluff, Taylortown, or certain sections within and north of Pinehurst, all of which are communities in southern Moore County, North Carolina. In addition, residents had to *a*) obtain their drinking water from a groundwater source, *b*) be able to speak English, *c*) have a listed telephone number, and *d*) not have worked in a pesticide manufacturing company (as we were interested in residential pesticide exposures). Residents in the communities surrounding Aberdeen were excluded if they had ever lived in Aberdeen. A detailed discussion of



**Figure 1.** Study areas with dump sites (A) Aberdeen; (B) and (C), comparison areas.

participant selection and enrollment for the phase I telephone survey study is presented by Arndt et al. (11).

Enrollment in phase II was limited to 302 participants (151 Aberdeen residents, 151 comparison area residents) because of cost constraints and because with that number we estimated we could detect a 25% difference in the CD4:CD8 ratio between residents of Aberdeen and the comparison communities with more than 80% power, assuming an  $\alpha$  of 0.05. In the previous pilot study of pet dogs, a 25% reduction in the CD4:CD8

ratio was noted among the dogs from Aberdeen compared to the dogs from nearby towns (17).

Participants were enrolled in the phase II study between September 1994 and March 1996. Potentially eligible residents were sent a letter explaining the study. The letter was followed by a telephone call to assess eligibility. If eligible, two appointments were scheduled: one to answer a 30-min telephone questionnaire and one to provide 45 mL blood and undergo a skin test at a local health clinic from 0700 to 0930 hr on a Tuesday morning.

The skin test was read 48 hr later. Participants signed consent forms before any laboratory procedures were performed. Study participants were paid \$20 for out-of-pocket expenses. This research was approved by the Institutional Review Boards at the University of North Carolina Schools of Public Health and Medicine.

We excluded from participation those individuals who had a bleeding disorder, who reported testing positive for the human immunodeficiency virus (HIV), who reported having eczema on the forearms, and who had

**Table 1.** Contaminants of concern in surficial soil and groundwater at the Aberdeen pesticides dumps site.

Chemicals	Farm Chemicals		Twin		Fairway six		Mclver		Route 211	
	Surficial soil ( $\mu\text{g}/\text{kg}$ )	Ground water ( $\mu\text{g}/\text{L}$ )	Surficial soil ( $\mu\text{g}/\text{kg}$ )	Ground water ( $\mu\text{g}/\text{L}$ )	Surficial soil ( $\mu\text{g}/\text{kg}$ )	Ground water ( $\mu\text{g}/\text{L}$ )	Surficial soil ( $\mu\text{g}/\text{kg}$ )	Ground water ( $\mu\text{g}/\text{L}$ )	Surficial soil ( $\mu\text{g}/\text{kg}$ )	Ground water ( $\mu\text{g}/\text{L}$ )
<b>Pesticides</b>										
4,4'-DDD	15,975	0.05	1,276	0.05			4,101	0.05	17,679	0.05
4,4'-DDE	645	0.05	2,335	0.05			1,400	0.05	8.5	0.05
4,4'-DDT	959,500	0.05	29,183	0.05			34,240	0.03	354,926	0.05
Aldrin	100		48	3.5						
$\alpha$ -BHC	57,574	16	35,198	10			80,158	0.02	648,930	0.02
$\beta$ -BHC	29,278	9	3,230	5			14,718	0.03	35,885	0.04
$\delta$ -BHC	37,145	6.9	12,449	18			5,974	0.02	5,431	0.03
Dieldrin	260	0.4	213							
Endosulfan II ( $\beta$ )		0.1	23							
Endrin		0.2								
Endrin ketone	27.0	0.4	11.2							
$\gamma$ -BHC	89,475	10	15,123	4.1			2,985	0.01	12,158	0.02
$\gamma$ -Chlordane	148									
Heptachlor	94		8.9							
Heptachlor epoxide										
Toxaphene	286,212		21,093				30,265		692,172	
<b>Solvents</b>										
1,1,1-Trichloroethane		388		100		7.3				
1,1-Dichloroethene						2				10
1,2-Dichloroethene		21								
<b>Benzene</b>										
Carbon disulfide		5,066								
Carbon tetrachloride										
Ethyl benzene		13,011		600						
Tetrachloroethene		750		9						
Toluene		846		36						
Trichloroethene		17								
Xylenes (total)		49,104		2,900						
1,2,4-Trichlorobenzene		9.8		22		20				
2,4-Dimethylphenol		38		6						
2-Methylnaphthalene		8.1								
Bis(2-ethylhexyl)phthalate										
Naphthalene		11								
<b>Metals</b>										
Antimony				430						
Arsenic	2,463,000		3,300							
Barium	194,000	100	21,195		9,434		54		8,600	
Beryllium		4.7								
Cadmium										
Chromium		325	10,824	9	15,035	183				20
Cobalt				6					7,100	
Copper	54,172,000	1,819	33,208	180	10,742					
Lead	9,854,000	5	4,700	9						
Manganese	78,000	3,162	7,293	210	18,448	167	10,000		1,200,000	60
Mercury			87		117				4,800,000	
Nickel	2,000	103		36		116			5,300	
Selenium										
Silver				22						
Vanadium			15,895		111,522				10,000	
Zinc	15,268,000	4,599	5,580	540	24,269		4,400			

Data from the U.S. Environmental Protection Agency (13).

had an asthma attack in the past year (which might make them more susceptible to an allergic reaction to the skin test); we also excluded residents from the comparison communities who had worked in Aberdeen for at least 20 hr/week during the past year. Potential participants were temporarily excluded if they reported having had an acute infection within the past month, or surgery, chemotherapy, radiation therapy, immunizations, or a blood transfusion or took immunosuppressive drugs within the previous 2 months. Women who were pregnant or lactating were also excluded. Eligibility criteria were reassessed during a reminder call the night before the clinic visit. Appointments were rescheduled when necessary.

We made at least 10 attempts to contact potentially eligible participants over a 3-month period including daytime, evening, and weekend times. Reasons for nonparticipation include the following: 188 had an ineligible address, had moved, or had a non-working phone number; 98 were ineligible based on participation criteria; 118 had another household member already enrolled; 439 refused; 38 could not be reached, and 417 were not contacted because we reached the goal of 302 total participants. Of the residents eligible for the phase I study, 38% of those contacted in Aberdeen and 37% in the comparison areas participated in the phase II study. The most common reasons for refusal to participate were lack of interest and inability to make the time commitment for a 30-min telephone interview and two clinic visits. Some of those who had declined to participate further after phase I may actually have been ineligible for phase II, making participation rates conservative estimates.

### Blood Drawing

A trained nurse drew 45 mL blood from each study participant into vacutainer tubes. The blood specimens were analyzed for organochlorine pesticide levels and indicators of immune competence including numbers and percentages of various types of white blood cells, immunoglobulin levels (IgA, IgG, IgE, IgM), and degree of mitogen-induced lymphoproliferative activity with three different mitogens. The blood specimens were also analyzed for the presence of DNA damage as indicated by the lymphocyte micronucleus assay.

### Organochlorine Levels

Plasma DDE levels for 301 participants were evaluated as part of an organochlorine pesticide panel that included *p,p*-DDT, *p,p*-DDE, *p,p*-DDD, *o,p*-DDT, *o,p*-DDE, *o,p*-DDD,  $\alpha$ -hexachlorocyclohexane ( $\alpha$ -BHC),  $\beta$ -BHC,  $\delta$ -BHC, lindane, hexachlorobenzene,  $\alpha$ -endosulfan,  $\beta$ -endosulfan, dieldrin,

endrin, *cis*-chlordane, *trans*-chlordane, oxy-chlordane, heptachlor, and heptachlorepoxide. Assay methods were developed by LabCorp (Burlington, NC) based on methods reported by Lopez-Avila et al. (18) and Saady and Poklis (19). Briefly, chlorinated pesticides in plasma were extracted from a methanol-deproteinated sample into hexane, and the extract was cleaned by C18 solid-phase extraction. The extract was injected into a gas chromatograph with dual capillary columns and dual electron capture detectors (Perkin-Elmer gas chromatograph; Perkin-Elmer, Norwalk, CT). Both columns had to indicate a positive result for a sample to be considered positive for a pesticide. LabCorp calibrated the gas chromatograph and associated data reduction equipment on each day of use, running positive and negative controls after every 10th sample. Positive controls contained known amounts of every analyte included in the analysis. Negative controls consisted of a serum matrix. The average recovery for all analytes in the assay was estimated to be 100.9%. The lower limit of quantification for the assay was 1 ppb (20).

The same panel of 20 pesticides was analyzed at a different laboratory (Research Triangle Institute, Research Triangle Park, NC) on duplicate specimens for 14 arbitrarily selected study participants over a 3-week period. Agreement between the two laboratories was excellent (correlation coefficient = 0.96).

### Complete Blood Cell Counts

A complete blood cell count was performed on 298 participants. Standardized LabCorp procedures with Westgard rules of quality control were used (21).

### Lymphocyte Phenotypes

Percentages of the following lymphocyte phenotypes were determined at the University of North Carolina Hospitals for 300 participants according to the methods described by Tamul et al. (22): CD3 (total T cells), CD3 positive/CD4 positive (CD4) (T helper cells), CD3 positive/CD8 positive (CD8) (T suppressor cells), CD16 (majority of natural killer cells), CD19 (total B cells), and CD56 (natural killer cells subset). Centers for Disease Control and Prevention (CDC) guidelines for quality control were followed (23). The University of North Carolina Hospitals established reference ranges based on laboratory values from 25 normal adults (24). The values represent the mean + 2 SDs.

### Immunoglobulin Levels

LabCorp determined levels of IgG, IgA, IgM, and IgE for 299 study participants. IgG, IgA, and IgM were assayed using a

quantitative turbidimetric method based on an antibody-antigen reaction adapted for a centrifugal analyzer (Roche Cobas Mira, Indianapolis, IN) with reference ranges determined by LabCorp.

IgE levels were analyzed in a one-step, solid-phase immunoassay using two highly specific monoclonal mouse antibodies to IgE (Ciba-Corning Total IgE; Ciba-Corning, Atlanta, GA) with the Roche Cobas Core IgE instrument. The coefficient of variation for low, medium, and high mean values was < 5% (25).

### Skin Test

A total of 297 adults (148 Aberdeen, 149 comparison areas) underwent a skin test using the Multitest CMI skin test (Pasteur Merieux Serums & Vaccines S.A., Lyon, France; distributed by Connaught Laboratories, Inc., Swiftwater, PA). Five subjects (3 from Aberdeen and 2 from the comparison areas) who reported having previous widespread or systemic reactions to skin tests or tetanus or diphtheria vaccinations were excluded from the skin test studies.

The Multitest CMI skin test consists of a disposable plastic applicator containing eight sterile test units preloaded with seven delayed hypersensitivity skin test antigens and a glycerine control. The seven antigens are tetanus toxoid, diphtheria toxoid, *Candida*, *Tricophyton*, *Streptococcus*, *Proteus*, and tuberculin. For this study, the tuberculin unit was detached from the applicator because *a*) increased rates of mycobacteria exposure in the South (where the study took place) would likely have increased reactions to the tuberculin test, and *b*) follow-up of false positive skin tests with further evaluation and treatment would have been unduly burdensome to the study participants.

A trained nurse applied the skin test on the inner forearm of each study participant between 0700 and 0930 hr and then read the skin test results 48 hr later. For each antigen site, the indurated area was measured to the nearest 0.5 mm across two diameters perpendicular to each other. The overall diameter of the indurated area was calculated as the mean of the two diameters. In normal individuals previously exposed to one of the antigens, an indurated area should occur on the forearm, indicating that the immune system recognized the antigen. A negative test result could mean that the immune system did not recognize the antigen or that the person had never been previously exposed. See Vine et al. (26) for more details regarding skin test methods.

### Mitogen Stimulation Assays

We performed mitogen stimulation assays on 260 study participants (131 from Aberdeen

and 129 from the comparison communities) as described by Wilson et al. (27) using three mitogens: PWM, CON-A, and PHA. [Reasons for unanalyzed specimens include unsatisfactory specimen (19; e.g., clotted); technical difficulties (8), and laboratory closed for vacation (16).] Blood samples were maintained at a constant temperature and kept under conditions established by the AIDS Clinical Trials Study Group (23) during transportation and were delivered to the laboratory within 6 hr of blood donation. Lymphocyte separation and cultures were begun shortly thereafter. Tritiated thymidine was added at 72 hr, and cells were harvested 18 hr later. Radioactive counts per minute (CPM) were quantitated using a Matrix 96 Direct Beta Counter (Packard Bioscience Company, Downers Grove, IL).

### Micronucleus Assay with Peripheral Blood Lymphocytes

Of the 302 study participants, results for the micronucleus assay were available for 254 participants (130 from Aberdeen and 124 from the comparison communities). [Reasons for unanalyzed specimens include unsatisfactory specimen (14); technical difficulties (11); and laboratory closed for vacation (23)]. We cultured the peripheral blood lymphocytes (PBLs) as described by Erexson et al. (28,29). All assays were cultured by one technician, and slides from 254 participants were read by three different scorers trained by one person. Scorers noted the total number of micronuclei per 1,000 binucleates as well as the percentage of cells that contained micronuclei. Scorers were unaware of the characteristics of the individuals whose slides they were scoring. Despite quality control efforts, one scorer tended to produce lower values than the other two scorers, requiring us to control for scorer in the statistical analyses.

### Telephone Questionnaire

A 30-min telephone questionnaire was administered by trained interviewers to all participants within the 2 weeks before their appointment at the clinic. The questionnaire assessed residential, recreational, and occupational exposures to pesticides, other chemicals, and metals. In addition, residents were asked about their residential history, sources of tap water, water consumption, dietary intake of caffeine, alcohol, fruits, vegetables, meat, and dairy products, smoking habits, medical history, reproductive history (women only), allergies, and perceived stress and perceptions concerning health risk due to environmental exposures in their neighborhood—for example, swimming in or eating fish caught in a local lake near the dump sites.

### Residential Address Matching

To calculate the distance from each residence to each of the six dump sites, we first automatically address-matched street addresses to the North Carolina Department of Instruction's Transportation Information Management System (TIMS; Raleigh, NC) geographic information files using Arc/Info (Environmental Systems Research Institute Inc., Redlands, CA), with 28% exact matches. Enlarged maps marked by study participants during their clinic visit allowed the transfer into Arc-Info of the exact locations for the remaining residential addresses. Geographic coordinates for the dump sites were obtained from the Agency for Toxic Substances and Disease Registry (Atlanta, GA).

### Statistical Analysis Methods

**Residential location and plasma organochlorine pesticide levels.** To determine whether residents of Aberdeen (and more specifically, those who lived near the dump sites) have higher levels of organochlorine pesticide levels in their blood than people living farther away, we calculated mean and median plasma DDE values by age, sex, distance from dump sites, and study area. Only DDE levels were assessed because only one individual (from Aberdeen who reported occupational exposure to pesticides) had a detectable level of any of the other organochlorine pesticides analyzed, namely, heptachlorepoide (2.0 ppb).

Analyses were conducted with and without excluding residents who reported having been employed in occupations that might have exposed them to organochlorine pesticides to assess the effects of residential versus occupational exposures. Residents were considered "occupationally exposed" if they currently lived on a farm (9 Aberdeen, 8 comparison areas), had ever worked as a farmer or farm worker (29 Aberdeen, 17 comparison areas), pesticide formulator, applicator or mixer (12 Aberdeen, 20 comparison areas) gardener or groundskeeper (16 Aberdeen, 14 comparison areas), or ever had another job that exposed them to pesticides (12 Aberdeen, 20 comparison areas). A total of 104 persons were excluded from analyses based on reported occupational exposure.

We performed multivariate linear regression analyses with backward elimination strategies using SAS version 6.12 (SAS Institute, Cary, NC) to determine whether plasma DDE levels were associated with distance of residences from the dump sites among Aberdeen residents only and to assess whether Aberdeen residents have higher DDE levels than residents of the comparison areas, controlling for potential confounders and effect modifiers. Potential

confounders for inclusion in the initial models were identified from the literature and from among the variables that were predictive of DDE levels among residents of Aberdeen and the comparison areas. Covariates were evaluated sequentially and retained if there was more than a 10% change in the coefficient(s) of interest when they were eliminated from the model. Covariates included the following variables: age; sex; breast-fed; income > \$20,000; meat, dairy, vegetable, and fruit consumption/day; tap water source (private well vs. community supply); grew up in Aberdeen; occupational exposure to pesticides; recreational exposure to the dump sites; amount of plain tap water consumed per day; high school graduate;  $\geq 10$  years in current home; cigarette smoking (pack-years); and employed fulltime.

**Residential location, immune markers, and micronuclei.** To assess associations between residential location and immune markers, residential location was defined two ways: residence in Aberdeen versus the comparison areas, and residence within a mile of any dump site versus greater than 1 mile. We compared mean levels of each of the immune markers (blood cell counts, lymphocyte phenotypes, immunoglobulins, micronuclei, and mitogen stimulation assay results) among residents of Aberdeen and the comparison areas using analysis of covariance controlling for confounders. Mean values for each of the immune markers were also compared to established normal values. The number of individuals who had values higher or lower than established normal values was also compared across study areas using chi-square tests. Adjusted least-squares estimates (30) comparing mean levels of each of the immune markers among residents of Aberdeen who lived within 1 mile from any site with all other residents living > 1 mile from any site (including residents in the comparison areas) were calculated using PROC GLM of SAS (SAS Institute), controlling for confounders. We calculated *p*-values for differences in means with a *t*-test.

Demographic characteristics, occupational data, diet, and lifestyle habits were evaluated as potential confounders of residence–outcome associations. A variable was considered a confounder if it was associated with both residential location and any of the outcome variables among the unexposed such that there was a 10% difference in the estimated mean value of the outcome between covariate categories. Only age (< 40, 40–49, 50–59,  $\geq 60$ ), sex, and pack-years (nonsmoker/ex-smoker, < 10, 10–38,  $\geq 39$ ) met these criteria. We did not adjust for medical history factors, as they may have been a consequence of immune dysfunction.

## Results

### Descriptive Characteristics of the Study Population

Aberdeen residents were, on average, 5 years younger and had lived in their homes and in town 3.6 years longer than residents of the comparison areas (Table 2). Aberdeen residents were significantly ( $p < 0.05$ ) more likely to be female, employed full time, born in North Carolina, concerned about the effects of environmental chemicals on health, have a child under 6 years of age, and report fair or poor health. They were also more likely to be employed in a job requiring physical exertion, consume meat, have had recreational exposures to the dump sites (e.g., ate fish from or swam in a local lake), and have used flea and tick products than residents of the comparison areas. They were less likely to be married, consume alcohol daily, have played golf, lived directly on a golf course, or have had their homes commercially treated for insects. Past medical history and medication use was not remarkably different between the two groups.

### Residential Location and Plasma DDE Levels

Age-adjusted mean DDE levels were significantly higher among Aberdeen residents than among residents of the comparison areas (Aberdeen: 4.05 ppb, range: 1–32 ppb; comparison areas: 2.95 ppb, range 1–24 ppb,  $p = 0.01$ ). Average DDE levels among all study participants under 40 years of age was similar (1.6 ppb). Among those 40–59 or  $\geq 60$ , residents of Aberdeen had higher DDE levels (40–59 years: 4.8 ppb,  $\geq 60$  years: 5.7 ppb) than those in the comparison areas (40–59 years: 3.2,  $\geq 60$  years: 4.7 ppb), whether or not individuals who reported having been employed in occupations that may have exposed them to pesticides were excluded. In fact, controlling for age, race, sex, and area of residence (Aberdeen vs. the comparison areas), there was no difference in mean plasma DDE levels between those who reported having been employed in occupations in which they may have been exposed to organochlorine pesticides (mean DDE = 3.4 ppb, SD = 0.37) and those who did not report working in such occupations (mean DDE = 3.5 ppb, SD = 0.26;  $p = 0.85$ ).

Figure 2 shows that most Aberdeen residents with high plasma DDE values (i.e.,  $\geq 5$  ppb, approximately the 80th percentile) lived within 1 mile of any dump site, but particularly within 1 mile of the Farm Chemicals site. (The Twin site was considered the same as the Farm Chemicals site because it is located across the street.) Aberdeen residents  $< 40$  years of age did not have plasma DDE values  $\geq 5$ . Among Aberdeen residents 40–59 years of age, 35% (20/57) of those who lived

within a mile of any site had plasma DDE levels  $\geq 5$  ppb versus 15% (3/20) of those who lived farther away ( $p < 0.1$ ). Fifty percent (14/28) of those who lived within a mile of the Farm Chemicals site had plasma DDE levels  $\geq 5$  ppb versus 18% (9/49) of those who lived farther away ( $p < 0.05$ ). There

were no differences in the percentages of residents  $\geq 60$  years of age with DDE values  $> 5$  ppb by residential location near the dump sites, although small numbers make differences difficult to evaluate.

Mean plasma DDE levels among Aberdeen residents living within a mile of the

**Table 2.** Descriptive characteristics of residents who lived in Aberdeen and the comparison areas.

	Aberdeen ( <i>n</i> = 151) No. (%)	Comparison areas ( <i>n</i> = 151) No. (%)	$\chi^2$ <i>p</i> -value
Age (years)			
< 40	56 (37)	39 (26)	
40–49	51 (34)	40 (26)	
50–59	27 (18)	20 (13)	
$\geq 60$	17 (11)	52 (34)	0.00
Sex (female)	103 (68)	86 (57)	0.04
Race (white)	138 (91)	143 (95)	0.26
Years in town			
< 5	26 (17)	42 (28)	
5–< 10	35 (23)	48 (32)	
10–< 15	21 (14)	14 (9)	
$\geq 15$	69 (46)	47 (31)	0.01
Years in home			
< 5	37 (25)	67 (44)	
5–< 10	45 (30)	51 (34)	
10–< 15	23 (15)	11 (7)	
$\geq 15$	46 (30)	22 (15)	0.00
Born in North Carolina	91 (60)	60 (40)	0.00
Born in Moore County	43 (28)	38 (25)	0.52
College graduate	55 (37)	52 (35)	0.75
Household income $> \$20,000$	126 (88)	136 (94)	0.06
Married	112 (74)	130 (86)	0.01
Child $< 6$ years of age	31 (21)	18 (12)	0.04
Consume alcohol daily	9 (6)	28 (19)	0.00
Take vitamins regularly	63 (42)	79 (52)	0.07
Current smoker	43 (28)	31 (21)	0.11
Pack-years			
Non/ex-smoker	108 (72)	120 (79)	
< 10	11 (7)	11 (7)	
10–38	21 (14)	12 (8)	
$\geq 39$	11 (7)	8 (5)	0.31
Employed full time	111 (74)	78 (52)	0.00
Self-reported fair or poor health	21 (14)	8 (5)	0.01
Believe illness from home chemicals (yes or maybe)	18 (12)	7 (5)	0.02
Very concerned about chemical hazards	65 (43)	41 (27)	0.00
Private well	35 (23)	27 (18)	0.25
Fruit $\geq 1$ serving/day	32 (21)	33 (22)	0.94
Vegetables $\geq 1$ serving/day	29 (19)	42 (28)	0.09
Dairy $\geq 1$ serving/day	59 (40)	52 (34)	0.36
Meat $\geq 1$ serving/day	97 (65)	76 (50)	0.01
Ever breast-fed (women)	49 (49)	47 (55)	0.40
Occupational exposure <sup>a</sup>	54 (36)	49 (32)	0.54
Recreational exposure to dump sites <sup>b</sup>	80 (53)	61 (40)	0.03
Ever played golf	43 (28)	87 (58)	0.00
Live on golf course	0 (0)	38 (25)	0.00
Commercial treatment of home, termites	67 (46)	75 (50)	0.52
Commercial treatment of home, other insects	49 (33)	67 (45)	0.03
Use insecticides in home	95 (63)	103 (69)	0.33
Used flea and tick products in past 2 months	49 (32)	30 (20)	0.01
Hours sweat/week during work $> 75$ th percentile <sup>c</sup>	47 (31)	29 (19)	0.02

<sup>a</sup>Occupational exposure to pesticides if yes to one of the following: live on farm, worked as gardener, worked as farmer/farm worker, pesticide formulator, mixer or applicator, or other pesticide workers. <sup>b</sup>Yes to one of the following: ever played golf at Pit Golf Links, ever visited dump sites, ever eaten fish caught at Pages Lake, swam or waded in Pages Lake, fished in Pages Lake. <sup>c</sup> $\geq 5$  hr/week at job doing physical activity that caused sweating.

Farm Chemicals site were statistically significantly higher than among Aberdeen residents living > 1 mile from the site controlling for age (5.22 vs. 3.01 ppb,  $p = 0.0026$ ). For no other site were there significant differences in the mean plasma DDE levels of residents who lived more than and less than 1 mile from the site. However, few residents lived within 1 mile of some sites, and some residents lived within 1 mile of more than one site (Table 3).

Women 40–59 years of age who lived within 1 mile of the Farm Chemicals site had higher mean plasma DDE levels than female residents who lived farther away (40–49 years: 6.6 vs. 2.5 ppb; 50–59 years: 10.5 vs. 3.8 ppb; Table 3). Assessing median versus mean values did not change conclusions. Linear regression analyses controlling for age, race, income, and having breast-fed showed that female Aberdeen residents 40–59 years of age who lived within 1 mile of the Farm Chemicals site had statistically significantly higher plasma DDE values than Aberdeen residents and comparison area residents who lived farther away ( $p < 0.05$ ). Fewer males than females who lived near the Farm Chemicals site participated in the study, and males tended to be younger than females, with none in the 50–59 year age range. Nonetheless, men 40–49 years of age who lived near the Farm Chemicals site tended to have higher average plasma DDE levels than men who lived farther away (4.4 ppb vs. 2.7 ppb; Table 3). Few Aberdeen residents  $\geq 60$  years of age participated in the study, although two 60-year-old women living near the Farm Chemicals site also had relatively high plasma DDE levels (8.5 and 22.1 ppb). Those residents 40–60 years of age who lived within 1 mile of the Farm Chemicals site before 1985 when the plant was in operation and before any remediation efforts took place had higher median DDE levels than those who moved there after 1985 (before 1985: men, 5.5 ppb,  $n = 5$ , mean age = 44 years;

women, 9.5 ppb,  $n = 11$ , mean age = 51 years; after 1985: men, 1.7 ppb,  $n = 3$ , mean age = 42 years; women, 4.6 ppb,  $n = 11$ , mean age = 50 years). Although women 40–59 years of age living near the McIver site had higher mean DDE levels than residents living farther away, most (5/7) also lived within 1 mile of the Farm Chemicals site (Table 3).

### Immune Marker Results among Residents of Aberdeen Versus Comparison Areas

**Blood cell counts, lymphocyte phenotypes, and immunoglobulin levels.** For the most part, results of the complete blood count, other lymphocyte phenotypic markers, and immunoglobulin levels did not differ between Aberdeen residents and residents of the comparison areas. However, the number of Aberdeen residents with a low percentage ( $< 8\%$ , the lower limit of the normal reference range) of CD16 cells, a marker for natural killer cells, was statistically significantly higher than the number of residents in the neighboring communities [30/150 (20%) vs. 13/150 (9%),  $p = 0.005$ ].

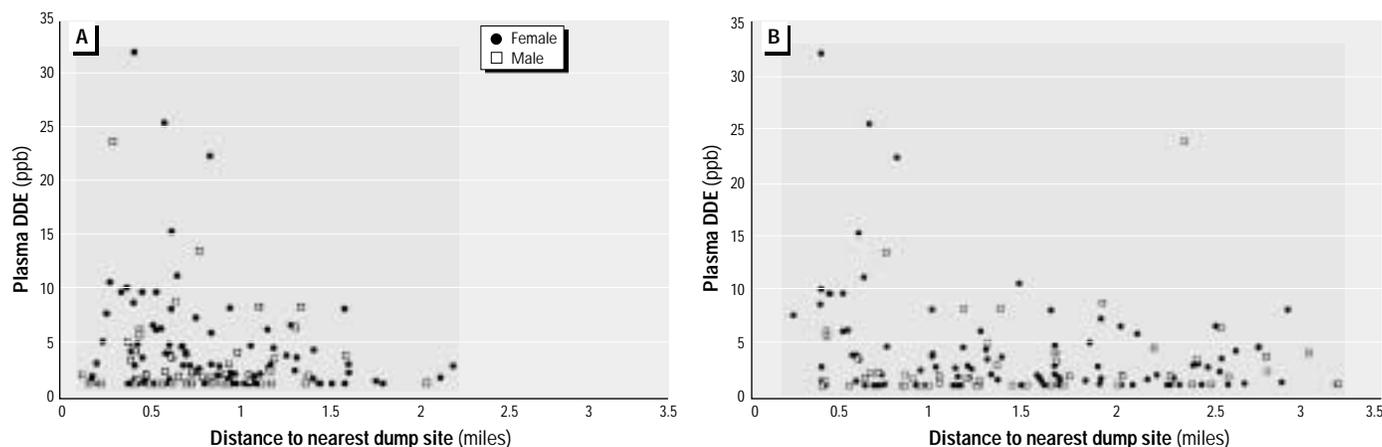
**Mitogen stimulation assay.** Crude analyses showed that mean counts on the mitogen stimulation assay were statistically significantly lower among Aberdeen residents as compared to residents in the comparison areas for PWM only (Table 4). Results were significantly lower for Aberdeen residents compared to residents of the comparison areas for CON-A and PWM ( $p < 0.05$ ) and suggestive for PHA ( $p = 0.08$ ) after controlling for age, sex, and pack-years (Table 4). Six women (four in Aberdeen and two in the comparison areas) had mitogen stimulation assay counts in the clinically suspect range (PHA  $< 20,000$ , CON-A  $< 15,000$ , PWM  $< 10,000$ ). Two of the Aberdeen residents lived within 1 mile of the Farm Chemicals site; one woman lived within 1 mile of another site, and the fourth did not live within a mile of any site.

As would be expected in a normal population, the results of the mitogen stimulation assays with the three different mitogens were highly correlated with one another: (PHA/CON-A,  $r = 0.73$ ,  $p = 0.0001$ , PHA/PWM logged to reduce skewness,  $r = 0.56$ ,  $p = 0.0001$ , CON-A/PWM logged,  $r = 0.69$ ,  $p = 0.0001$ ).

**Micronucleus assay.** There were no differences in micronucleus frequencies between the residents of Aberdeen and those in the comparison areas controlling for age, sex, pack-years, and scorer (e.g., mean percent of cells with micronuclei: Aberdeen, 1.49, SE = 0.077; comparison areas: 1.40, SE = 0.079;  $p = 0.40$ ).

### Immune Marker Results Among Residents Based on Proximity to the Dump Sites

**Blood cell counts, lymphocyte phenotypes, and immunoglobulins.** An immunosuppressive effect of living within a mile of any site was not supported by the results of the white blood cell counts, lymphocyte phenotypic marker assays, or the immunoglobulin assays after controlling for age, sex, and pack-years. There was a suggestion of a decrease in CD4 cells (percentage of total lymphocytes,  $p = 0.08$ ) and an increase in CD8 cells (total,  $p = 0.09$ ; percent of total lymphocytes,  $p = 0.07$ ) among those living closer to the sites. This resulted in a slight drop in the CD4:CD8 ratio among those within a mile of any site (2.2 vs. 2.0,  $p = 0.13$ ). The drop in the CD4:CD8 ratio was greater among those who lived farther than a mile from the Farm Chemicals site compared to those who lived within a mile of the site (all ages: 2.2 vs. 1.9,  $p = 0.05$ ; 40–59 year olds, 2.4 vs. 1.9,  $p = 0.10$ ). Of the immunoglobulins, only IgM appeared to be statistically significantly associated with living within 1 mile of any dump site compared to living farther away. IgM was statistically significantly higher among residents living close to the sites whether or not



**Figure 2.** Plasma DDE levels (ppb) by (A) distance to nearest dump site (miles) among Aberdeen residents and (B) distance to Farm Chemicals (miles) among Aberdeen residents.

we controlled for age, sex, and pack-years (143 vs. 123 mg/dL,  $p = 0.03$ ).

**Mitogen stimulation assay.** All three mitogen stimulation assay results were lower among residents who lived within a mile of any dump site as compared with residents who lived farther away. The results were statistically significant for PWM after controlling for age, sex, and pack-years ( $p = 0.05$ ; Table 5).

**Micronuclei.** Neither the total number of micronuclei nor the percentage of cells with micronuclei was associated with living within a mile of any dump site, controlling for age, sex, and pack-years. However, among those 40–59 years of age, both the number of micronuclei/1,000 binucleate cells and the percentage of cells with micronuclei were statistically significantly elevated among those who lived within a mile of the Farm Chemicals site compared to those who lived farther away (number of micronuclei/1000 binucleate cells < 1 mile of Farm Chemicals:

21,  $n = 25$ ; > 1 mile from Farm Chemicals: 16,  $n = 92$ ,  $p = 0.04$ ; percentage of micronuclei < 1 mile from Farm Chemicals: 1.9, > 1 mile from Farm Chemicals 1.4,  $p = 0.04$ ).

### Skin Test Results

Overall, residents of Aberdeen and the comparison areas did not differ with regard to the percentage of individuals with positive skin test results by sex (Table 6). Multivariate logistic regression modeling controlling for age and household income > \$20,000 confirmed these results. The number of positive responses was too small to assess effects of skin testing by distance from each dump site.

## Discussion

### Summary of Results

Of 20 organochlorines tested, only DDE was detected in the blood of participants (except for one individual with heptachlor epoxide

levels of 2 ppb). Age-adjusted mean plasma DDE levels were 4.05 ppb for Aberdeen residents and 2.95 ppb ( $p = 0.01$ ) for residents of neighboring communities. Residents ages 40–59 years who lived within a mile of any site, but particularly the Farm Chemicals site, had higher plasma DDE levels than residents who lived farther away. Residents who lived within a mile of the Farm Chemicals site before 1985 when the plant was in operation and before any remediation efforts took place also had higher plasma DDE levels than residents who lived there after 1985.

Overall, there were few differences in immune markers between residents of Aberdeen and the neighboring communities. When comparing Aberdeen residents to residents of the comparison areas, we noted that a) the number of Aberdeen residents with a low percentage (< 8%) of CD16 cells, a marker for natural killer cells, was statistically significantly higher than the number of residents

**Table 3.** Mean (SE) plasma DDE levels (ppb) by distance from each dump site, age, and sex.

Dump site	Sex	Age (years)	≤ 1 mile				> 1 mile			
			No.	Mean (ppb)	SE	Range (ppb)	No.	Mean (ppb)	SE	Range (ppb)
Farm Chemicals (Twin)	M	< 40	7	1.3	0.16	1–2.1	27	1.6	0.24	1–6.8
		40–49	8	4.4	1.4	1–13.3	25	2.7	0.40	1–8.0
		50–59	–	–	–	–	20	5.3	1.2	1–23.5
		≥ 60	1	1.0	–	1–1.0	25	4.2	0.52	1–8.8
	F	< 40	7	1.2	0.14	1–2.0	54	1.8	0.13	1–4.7
		40–49	12	6.6	1.9	1–25.2	45	2.5	0.33	1–10.4
		50–59	8	10.5	3.5	1.4–31.8	19	3.8	0.62	1–8.9
		≥ 60	4	8.9	4.7	1–22.1	39	5.1	0.68	1–23.9
	Total		47				254			
	Mclver	M	< 40	1	1.4	–	1.4–1.4	33	1.5	0.20
40–49			1	1.0	–	1–1.0	32	3.2	0.47	1–13.3
50–59			2	5.3	3.4	1.9–8.6	18	5.3	1.3	1–23.5
≥ 60			1	1.0	–	1–1.0	25	4.2	0.52	1–8.8
F		< 40	5	1.3	0.20	1–1.9	56	1.7	0.13	1–4.7
		40–49	3	5.7	2.3	1.5–9.5	54	3.3	0.53	1–25.2
		50–59	4	13.4	6.3	3.8–31.8	23	4.5	0.79	1–15.1
		≥ 60	2	4.2	1.5	2.7–5.7	41	5.5	0.78	1–23.9
Total			19				282			
Route 211		M	< 40	3	1.3	0.27	1–1.8	31	1.5	0.21
	40–49		1	1.8	–	1.8–1.8	32	3.2	0.47	1–13.3
	50–59		2	12.3	11.3	1–23.5	18	4.6	0.70	1–12.2
	≥ 60		2	4.2	0.25	3.9–4.4	24	4.0	0.56	1–8.8
	F	< 40	7	1.6	0.25	1–2.9	54	1.7	0.13	1–4.7
		40–49	5	2.6	0.85	1–4.9	52	3.5	0.56	1–25.2
		50–59	4	5.0	1.5	1–7.9	23	5.9	1.4	1–31.8
		≥ 60	–	–	–	–	43	5.5	0.74	1–23.9
	Total		24				277			
	Geigy	M	< 40	5	1.2	0.16	1–1.8	29	1.6	0.23
40–49			7	4.6	1.5	1.8–13.3	26	2.8	0.41	1–8.0
50–59			4	6.8	5.6	1–23.5	16	5.0	0.73	1.7–12.2
≥ 60			1	4.4	–	4.4–4.4	25	4.0	0.54	1–8.8
F		< 40	17	1.9	0.22	1–4.0	44	1.6	0.14	1–4.7
		40–49	8	3.3	1.1	1–10.4	49	3.4	0.57	1–25.2
		50–59	5	2.8	1.1	1–6.4	22	6.5	1.5	1–31.8
		≥ 60	3	9.9	6.1	2.8–22.1	40	5.1	0.68	1–23.9
Total			50				251			

No one lived within 1 mile of Fairway six; 8 out of 19 who lived within 1 mile of Mclver also lived within 1 mile of Farm Chemicals; 0 out of 24 who lived within 1 mile of Route 211 also lived within 1 mile of Farm Chemicals; and 7 out of 50 who lived within 1 mile of the Geigy site also lived within 1 mile of Farm Chemicals.

in the neighboring communities (20% vs. 9%,  $p=0.005$ ), and *b*) the results of the mitogen stimulation assay were statistically significantly lower among Aberdeen residents compared to residents in the comparison areas (CON-A,  $p=0.02$ ; PWM,  $p=0.002$ ). Aberdeen residents who lived within a mile of any dump site compared to all other study participants also had lower mitogen stimulation assay counts PWM,  $p=0.05$ ). IgM levels were statistically significantly higher among residents who lived within a mile of any dump site compared to residents who lived farther away (143 vs. 123 mg/dL,  $p=0.03$ ). Those who lived within a mile of the Farm Chemicals site had a lower CD4:CD8 ratio than residents who lived farther away (1.9 vs. 2.2,  $p=0.05$ ). CD4 tended to decrease, while CD8 tended to increase. Residents 40–59 years of age, who lived within a mile of the Farm Chemicals site had a higher percentage of cells with micronuclei than residents who lived farther away (1.9% vs. 1.4%,  $p=0.04$ ). There were no consistent differences in response to the skin tests by residential location.

### Plasma DDE Levels and Residential Location

It is not surprising that residents near the Farm Chemicals site had the highest levels of DDE (a metabolite of DDT) in their blood because, of the six sites, Farm Chemicals had the greatest amount of DDT contamination (13). Levels of plasma DDE in the study population overall were low as compared to nationwide levels measured during the late 1970s, shortly after the 1972 ban of DDT use in the United States. According to data from the Second National Health and Nutrition Examination Survey (NHANES II) (34), median DDE levels were 12.0 ppb and 18.3 ppb for participants ages 25–44 and 45–74, respectively. More recent nationwide values are not available. A comparison of overall plasma DDE levels between residents of Moore County in this study and women in 24 counties in North Carolina from another recent study showed that DDE levels were of similar magnitude (32).

Organochlorines such as DDE are stored in adipose tissue for a long time. Blood levels are considered good indicators of DDE body burden, as adipose tissue and blood levels have been shown to be highly correlated (DDE:  $r=0.95$ ) (33). If current DDE exposure is not as high as past exposure, there may be some movement of the DDE from the fat tissue into the blood. Therefore, current plasma DDE levels reflect past as well as current exposures. The fact that residents who lived within a mile of the Farm Chemicals site before 1985 had higher median DDE levels than those who moved there after 1985 suggests that exposure levels were

higher in the past. In the phase I telephone survey, we found that residents who lived in Aberdeen before 1985 had a 2- to 3-fold higher risk of herpes zoster (which we used as an indicator of immune suppression) than residents of the comparison communities.

In previous studies, age, sex (34), race (35), breast-feeding, diet (largely fat consumption), and smoking (36) have been associated with increased plasma DDE levels. In this study, all of these factors were associated with increased DDE levels (12).

**Table 4.** Mean ( $\pm$  SD) of mitogen stimulation assay results by study area.

Mitogens	Aberdeen mean $\pm$ SD ( $n=131$ )	Comparison mean $\pm$ SD ( $n=129$ )	$p$ -Value	Reference ranges <sup>a</sup>
PHA				
Crude	131,710 $\pm$ 38,583	133,563 $\pm$ 38,274	0.70	> 71,969
Adjusted <sup>b</sup>	128,530 $\pm$ 3,197 <sup>c</sup>	136,792 $\pm$ 3,223 <sup>c</sup>	0.08	> 71,969
CON-A				
Crude	67,025 $\pm$ 23,716	71,508 $\pm$ 24,636	0.14	> 31,328
Adjusted <sup>b</sup>	65,776 $\pm$ 2,108 <sup>c</sup>	72,776 $\pm$ 2,126 <sup>c</sup>	0.02	> 31,328
PWM				
Crude	34,714 $\pm$ 20,845	39,930 $\pm$ 22,063	0.05	> 16,666
Adjusted <sup>b</sup>	33,068 $\pm$ 1,878 <sup>c</sup>	41,601 $\pm$ 1,893 <sup>c</sup>	0.002	> 16,666

<sup>a</sup>Reference ranges indicate top 95% of the values of the University of North Carolina Hospital normal controls.

<sup>b</sup>Controlling for age, sex, and pack-years. <sup>c</sup>Standard error.

**Table 5.** Mitogen stimulation assay results by distance from any dump site.

Outcome	$\leq 1$ mile from any site ( $n=90$ )		$> 1$ mile from any site ( $n=170$ )		$p$ -Value <sup>a</sup>
	Mean	SE	Mean	SE	
PHA					
Crude <sup>b</sup>	131,805	4,102	133,065	2,928	0.80
Adjusted <sup>c</sup>	129,874	3,863	134,087	2,773	0.38
CON-A					
Crude <sup>b</sup>	66,745	2,435	70,575	1,900	0.23
Adjusted <sup>c</sup>	66,024	2,549	70,956	1,830	0.12
PWM					
Crude <sup>b</sup>	34,832	2,298	38,610	1,641	0.18
Adjusted <sup>c</sup>	33,567	2,283	39,278	1,640	0.05

<sup>a</sup> $p$ -Value for the statistical significance of the difference in mean values. <sup>b</sup>Crude  $p$ -values calculated by *t*-test. <sup>c</sup>Adjusted for age, sex, and pack-years.

**Table 6.** Number and percentage of individuals with a positive ( $\geq 2$  mm) skin test by study area and sex.

Skin test	Aberdeen No. (%)	Comparison areas No. (%)	$p$ -Value <sup>a</sup>
At least one positive skin test			
Males	35 (74)	55 (85)	0.18
Females	59 (58)	44 (52)	0.41
At least two positive skin tests			
Males	20 (43)	36 (55)	0.18
Females	27 (27)	18 (21)	0.40
Tetanus			
Males	28 (60)	48 (74)	0.11
Females	45 (45)	33 (39)	0.47
Diphtheria			
Males	14 (30)	24 (37)	0.43
Females	19 (18)	15 (18)	0.90
Streptococcus			
Males	8 (17)	10 (15)	0.82
Females	6 (6)	4 (5)	0.73
Candida			
Males	4 (9)	18 (28)	0.01
Females	7 (7)	5 (6)	0.78
Trichophyton			
Males	4 (9)	6 (9)	0.90
Females	1 (1)	1 (1)	0.90
Proteus			
Males	13 (28)	21 (32)	0.60
Females	24 (24)	13 (16)	0.16

Numbers of participants: Aberdeen, males, 47, females, 101; comparison areas, males, 65, females, 84.

<sup>a</sup> $p$ -Values calculated by chi-square test.

## Route of Exposure

Although groundwater was a primary concern as a route of human exposure to the contents of the dump sites, it is not likely that groundwater was responsible for the majority of the higher blood levels of DDE among Aberdeen residents. According to county groundwater flow charts (37), no pesticide contamination was expected in the groundwater in the area to the northeast of Farm Chemicals, where most of the people with plasma DDE levels  $\geq 5$  ppb lived. Current water consumption was not predictive of plasma DDE levels among individuals who lived near the Farm Chemicals site. The majority of people in that area received their tap water from the Aberdeen municipal water company (which derives its water from numerous wells in and around the town) and not from private wells. Similarly, groundwater flow near the other dump sites suggests that groundwater was not primarily responsible for the higher blood levels of DDE.

As Mobbs (16) explained in his 1948 letter, to protect workers at the Farm Chemicals plant, exhaust fans blew pesticide dusts that spilled during the bagging process out into the surrounding area, making air a likely route of exposure. Other sources of exposure may have included direct contact with the sites or eating contaminated fish from a nearby lake.

## Organochlorines and Immune Markers

In the current study, a greater number of Aberdeen residents had low ( $< 8\%$ ) CD16 cells, a marker for natural killer cells, than residents of the comparison areas. Animal studies have noted a reduction in natural killer cell activity with exposure to organochlorines (38). Natural killer cells are involved in host defense against viral infections and possibly malignancies. Residents who lived within 1 mile of the dump sites had higher IgM levels than residents who lived farther away. Studies assessing the relationship between organochlorine exposure and immunoglobulin levels have produced conflicting results (12).

As in Backer's study of pet dogs (17), there was a suggestion of a decrease in the CD4:CD8 ratio in association with living near the dump sites, especially the Farm Chemicals site (2.2 for  $> 1$  mile from Farm Chemicals vs. 1.9 for  $< 1$  mile from Farm Chemicals,  $p = 0.05$ ). The literature on human studies contains mixed results. Workers exposed to chlorinated pesticides among other pesticides also experienced depressed CD4:CD8 ratios (males: controls,  $n = 19$ , 1.98; workers,  $n = 19$ , 1.74,  $p = 0.02$ ; females: controls,  $n = 18$ , 2.07; workers,  $n = 18$ , 1.63,  $p = 0.001$ ) (39). On the

other hand, individuals exposed to the termiticide chlordane at home or at work had a nonstatistically significant increase in their CD4:CD8 ratio (exposed 2.2,  $n = 27$ ; controls, 1.9,  $n = 114$ ) (40). Alterations in the CD4:CD8 ratio and antibody responses were the most predictive markers of the immunotoxicity of various chemicals in mice (41).

The results of the current study show an association between living near the dump sites and decreased mitogen-induced lymphoproliferative activity. McConnachie and Zahalsky reported an association between organochlorine exposure (chlordane) and decreased response to mitogen stimulation (PHA, CON-A, PWM) among people exposed at home or at work 2–10 years before their study. DDT has also been found to inhibit human lymphocyte PHA mitogenesis in occupationally exposed workers (42). Furthermore, blood levels of DDT and DDE were inversely correlated with PHA and CON-A mitogenesis in free-living bottlenosed dolphins (43).

Lowered mitogen-induced lymphoproliferative activities indicate that both T and B cells are less responsive to stimulation by particular mitogens and, therefore, suggest that they are less able to initiate or augment an immune response to foreign agents. In states where there is substantial suppression of T and B cell function, there is increased susceptibility to infection (e.g., by viral and fungal agents) and to cancer.

As per the results of previous studies, factors associated with immune markers in the current study included age (44), sex (45), and smoking (46–48), although not every marker was associated with all three factors. Each factor was included as a potential confounder in analyses for consistency of reporting.

A statistically significant association between living near the Farm Chemicals site and higher micronucleus frequencies was noted among residents 40–59 years of age. Backer (17) noted a statistically significant elevation in the percentage of cells with micronuclei among dogs in Aberdeen compared to those in neighboring communities. Other investigators have found some organochlorines including DDT to be mutagenic (49–51).

## Skin Test Results

No consistent differences in skin test positivity were found in association with residential location (Aberdeen vs. the comparison areas) evaluated separately by sex, controlling for age, race, income, and pack-years. There are few studies of this type with which to compare results. One study of Brazilian children exposed to DDT found no association between exposure and diphtheria immunization response (52). Street and Sharma (53)

noted a decreased delayed-type hypersensitivity skin test response to tuberculin in rabbits exposed to DDT. Small numbers prevented adequate assessment of skin test reactivity by residential distance from the dump sites.

Bernstein and Storms (54) suggested that the Multitest device may not be as sensitive a measure of delayed-type hypersensitivity as intradermal testing methods. They report that in some studies, it has taken 96 hr for responses to occur using the Multitest CMI skin test that took only 48 hr to appear using intradermal testing methods. It is possible that the lower response rate using the Multitest device could have led to an underestimate of the effects of exposure on skin test reactivity.

## Limitations of the Study Design

This cross-sectional study was designed to assess the relationship between location of residence and markers of immune competence at one point in time. Thus, individuals who may have been affected by the chemicals in the dump sites could have moved or died before the study, and any association that may have existed between earlier exposure to the dump sites and the various immune marker outcomes may have been missed.

Furthermore, the immune system changes with time. Immune cells, which are among the shortest lived cells in the body, constantly turn over and are replaced by other cells. Exposures that occurred many years ago may have affected the immune systems of residents at that time, but those alterations are unlikely to be detectable at present. For example, immune changes returned to normal within 3 months among a group of occupationally exposed pesticide workers who left employment (55).

Another possible limitation of the study is that certain effects of exposure to the dump sites may be observable only in susceptible populations, such as young children who were not included in this study. Soviet studies indicate that children are particularly sensitive to pesticide-induced immunosuppressive effects (4).

Although the dump sites contained volatile organic compounds and metals as well as organochlorines, only organochlorines were measured in blood because they were the contaminants of greatest interest at the sites. Therefore, residential distance from the dump sites was used as a proxy measure of exposure to the sites in analyses with immune markers as outcome measures.

Multiple statistical comparisons could account for random inconsistent positive study results such as the greater number of Aberdeen residents with a low percentage of natural killer cells (CD16 cells) and increased IgM levels, and inconsistencies in skin test

results. Uncontrolled confounding by other environmental contaminants may also have accounted for some positive results. For example, we did not measure plasma PCB levels because they were not considered contaminants of the sites.

Although participation rates in the original phase I telephone survey study were about average for telephone surveys (Aberdeen 71%, comparison areas 62%), the participation rate in phase II was lower than desirable, at about 37%. Clinic study participants differed from telephone survey participants in that they were more likely to be female, white, educated, of higher income (income > \$30,000) and have had recreational exposures to the dump sites. Phase II participants from the comparison areas tended to be older and more healthy than telephone survey study participants.

### Strengths of the Study Design

This study is the first to extensively evaluate the effects on the immune system of living near a hazardous waste site containing organochlorine pesticides. Participants for phase II were randomly selected from among those who participated in the original Phase I telephone survey study. Participants were screened to exclude individuals with temporary disturbances in immune markers (e.g., those with acute illnesses and those on medications that affect the immune system). Participants from Aberdeen and the surrounding communities were enrolled in the study simultaneously. Rigorous quality control measures ensured reliable questionnaire and laboratory data. Many potential confounders were considered in the study design and analysis. Because groundwater was a concern as a possible route of exposure, many questions concerning source of tap water and amount of water consumed were evaluated. All study participants received their tap water from groundwater sources. There was ample power to determine whether residents of Aberdeen differed from residents of the comparison areas with respect to immune markers. For example, we could detect a 16% difference in the CD4:CD8 ratio with 80% power.

### Overall Conclusions

Despite the large number of pesticides contained in the dump sites, of a panel of 20 organochlorines, DDE was the only organochlorine detected in the blood of 302 study participants (except for one individual with heptachlor epoxide levels of 2 ppb). Aberdeen residents ages 40–59 who lived near the dump sites, and in particular, near the Farm Chemicals site, had higher plasma DDE levels compared to Aberdeen residents and residents of the comparison areas who lived farther away. Levels of plasma DDE in

the study population overall were low as compared to nationwide levels between 1976 and 1980, but similar to levels in a concurrent study in North Carolina. Although some statistically significant differences in immune markers were noted with respect to location of residence near the dump sites, the magnitude of the effects are of uncertain clinical significance. Most individuals had marker levels well within normal ranges. It is possible that higher level exposures in the past resulted in immune marker changes that were not detectable in the current study due to the recovery of the immune system. In fact, residents who lived within a mile of the Farm Chemicals site before 1985 when the plant was in operation and before any remediation efforts took place had higher median DDE levels than those who moved there after 1985. Soil and groundwater remediation efforts which have already taken place should limit further exposure to the contents of the dump sites.

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